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Bisphenol A-induced downregulation of murine macrophage activities in vitro and ex vivo

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Abstract

Bisphenol A (BPA) is known to have detrimental effects on the reproductive system, but the toxicity of BPA on immune responses has not been systematically investigated. We investigated the effects of BPA exposure on the activities of murine peritoneal macrophages through evaluation of BPA-induced alteration of nitric oxide (NO) production, tumor necrosis factor-α (TNF-α) synthesis, and expression of co-stimulatory molecules B7. Macrophages were examined ex vivo from mice orally treated with various doses of BPA for 5 consecutive days per week for 4 weeks followed by culture for 2 or 4 days in the presence of lipopolysaccharides (LPS). Macrophages from naive mice were also stimulated with LPS ± BPA for 2 or 4 days. NO production was decreased with the in vitro exposure to 1, 10 and 100 μM BPA. NO production was lower in the BPA-exposed mice than the control mice with all doses. In vitro, BPA suppressed TNF-α secretion with significant reduction at 10 and 100 μM BPA. Similar findings were observed with the macrophages from the BPA-exposed mice. This study provides the substantial evidence on BPA-induced alteration in macrophage activity.
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Keywords: Bisphenol A; Macrophages; Nitric oxide; Tumor necrosis factor-α; B7.

1. Introduction

Bisphenol A (4,4′-isopropylidine-2-diphenol) (BPA) has been considered an endocrine disrupter (Palanza et al., 2002). BPA is a monomer used in manufacturing epoxy resins and polycarbonates, and humans may be occupationally or environmentally exposed to it. There could be potential for oral exposure to trace amounts of BPA since the epoxy resins or polycarbonates are materials used in producing portable containers for food or water as well as dental sealant (Brotons et al., 1995; Mountfort et al., 1997). BPA has been extensively studied especially concerning reproductive and developmental toxicity (Khurana et al., 2000; Morrissey et al., 1987). The estrogenic potential of BPA has been reported (Bergeron et al., 1999; Gould et al., 1998; Markey et al., 2001; Olea et al., 1996). BPA was also reported to cause allergic contact dermatitis (Estlander et al., 1999), in which antigen presenting cells, including macrophages could be involved with occurrence of the disease (Kiekens et al., 2001). However, the effects of BPA on macrophage activities have not been systematically evaluated. Environmental estrogens such as genistein, 4-octylphenol, or estradiol have been reported to modulate the function of macrophages, resulting in suppression (Deshpande et al., 1997; Inadera et al., 2000; Salem et al., 1999, 2000) or enhancement (Ruh et al., 1998) of its activities probably through estrogen receptor (Gulshan et al., 1990). Herein, we investigated the immunotoxicological effects of BPA exposure on activation of murine macrophages through analysis of NO production, TNF-α synthesis, and expression of CD80 (B7-1) and CD86 (B7-2) following BPA exposure in vitro and in vivo.

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2. Materials and methods

2.1. Animals

Female ICR mice, 3 weeks of age, were obtained from Yuhan Corporation in Korea where mice have been managed under the Korean Government Guideline on specific pathogen-free animal facility. Mice were acclimated for 3 weeks prior to use in our experiments. They were housed in sterile laminar flow cages in a specific pathogen free facility of the Sookmyung Women's University and maintained on mouse chow and acidified water ad libitum. Mice were weighing 26 ± 2 g at the beginning of the experiments.

2.2. Medium and reagents

BPA and corn oil were purchased from Sigma (St. Louis, MO, USA), and Dulbecco's modified Eagle's medium (DMEM, Gibco-BRL, Grand Island, NY, USA) was supplemented with non-essential amino acids (1%), sodium pyruvate (1 mM), sodium bicarbonate (0.044 mM) from Biowhittaker (Walkersville, MD, USA), antimycotic-antibiotics (1% mM) and heat-inactivated fetal bovine serum (10%, FBS, Gibco-BRL). LPS derived from *Escherichia coli* was purchased from Sigma and recombinant IFN-γ from BD PharMingen (San Diego, CA, USA). Anti-CD80, -CD86, -CD 16/32 mAbs, and TNF-α detection kit were purchased from BD PharMingen. Anti-F4/80 Ab was purchased from Serotec (Kidlington, UK).

2.3. Treatment with BPA and preparation of peritoneal macrophage

BPA was dissolved in corn oil and administered through oral route at a dose of 100, 500, or 1000 mg/kg body weight/day for 5 consecutive days per week for 4 weeks. The BPA doses were based on the results of the developmental toxicity evaluation demonstrating 640 mg/kg as the developmental NOAEL in mice (Morrissey et al., 1987; NTP, 1985). Control mice were administered with the same volume of coin oil. Five mice were used for each dose per experiment and the experiment was performed three times. For evaluation of in vitro BPA effects, peritoneal macrophages from naive mice untreated with BPA were exposed to DMEM with 1% DMSO containing 1, 10 and 100 nM, and 1, 10, and 100 μM BPA. Mice were sacrificed and injected intraperitoneally with 8 ml cold DMEM media, thereafter, peritoneal fluid was withdrawn using a 20 ml syringe. The peritoneal fluid was centrifuged 5 min at 800 × *g* at 4 °C. Red blood cells (RBC) were lysed with cold 0.2% NaCl. The remaining cells were washed with cold PBS, resuspended in DMEM media containing 10% FBS, and adjusted to 4×10^6 cells/ml. The cells were consisting of ≥89% F4/80$^+$ cells. F4/80 is an antigen expressed by almost all murine macrophages (Lee et al., 1985). Tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed as described previously (Mosmann, 1983) to examine cell viability following in vitro supplementation of BPA.

2.4. NO and TNF-α determination

The peritoneal exudates minus RBC (2×10^6 cells/ml per well) were placed into wells of 24-well culture plate for 2 h in CO_2 incubator (37 °C) for adherence isolation of macrophages. After washing out non-adherent cells, the remaining peritoneal macrophages were stimulated with 1 ml DMEM culture medium in the presence of 1 μg/ml LPS for NO production. This assay was performed for assessment of ex vivo and in vitro analysis of BPA. After 4 days incubation, culture supernatants were collected for NO measurement. The effect of BPA on NO production was evaluated through measurement of nitrite (NO^{2-}), a stable oxidative end product of NO. The method for measuring nitrite was performed as described previously (Green et al., 1992). Briefly, 100 μl of culture supernatants was mixed with 100 μl of Griess reagent (1:1 mixture of 2% sulfanilamide and 0.2% napthylethylenediamine dissolved in 5% phosphoric acid, respectively) in the wells of a flat-bottom 96-well plate. The plate was incubated on a shaker for 10 min at room temperature and read using a microplate reader (Bio-Tek ELx800, Burlington, VT, USA) at 570 nm.

Stimulation protocol for evaluation of BPA effects on TNF-α production from peritoneal macrophages was the same as for the NO production except analysis was performed 2 days after stimulation. The amounts of TNF-α in the supernatants were assayed by a sandwich ELISA using a kit from BD PharMingen. The lower limit of detection was 20 pg/ml.

2.5. Expression of B7-l and B7-2 molecules

Peritoneal macrophages (1×10^6 cells/ml) were suspended in PBS containing 5% FBS and 0.1% sodium azide. Non-specific binding of fluorescence antibody was blocked by incubating with 1 μg CD16/CD32 Fc Block™ (BD PharMingen) for 30 min, which binds to Feγ III/II receptors on macrophages. Phycoerythrin (PE) anti-B7-1 or PE anti-B7-2 was then added and incubated for an additional 30 min in the presence of fluorescein isothiocyannate (FITC) anti-F4/80. Cells were washed three times with PBS supplemented with 5% FBS and 0.1% sodium azide, and analysed on the flow cytometer (FACSCalibur™, BD Biosciences, Frankin Lakes, NJ, USA).

2.6. Statistical analyses

Data were initially evaluated for normal distribution. Statistical significances among groups were tested using Sigmaplot (SPSS Inc., Chicago, IL, USA) by single-factor ANOVA and Dunnett's *t*-test or Kruskal–Wallis ANOVA and Dunn's test, depending on normality of data. The significances were further confirmed by Student's *t*-test or

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